

# Extrapolation of Experimental Data from Animals to Man

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Conditions for extrapolating toxicologic data from animals to man were studied. In the search for general regularities associated with the comparative sensitivity of humans and various species of animals to toxins, it was shown that the toxicity parameters of compounds and the biological constants of mammals correlate with body weight. This relationship is well described by a rectilinear regression equation which holds for more than 100 of the most diverse mammalian biological constants. The toxicity parameters for 80% of the substances also are subordinated to this regularity. This made it possible to develop a computational method for extrapolating toxicologic data from animals to the "average" man. In order to increase the reliability of extrapolation, it is necessary to take into consideration the limits of variability of sensitivity of various population contingents to the effect of chemical compounds, estimate the accuracy of establishing the threshold and no-effect doses of substances under chronic experiment conditions with animals and determine the maximally possible error associated with extrapolating experimental data to the "average" man. In this respect, it is advisable to use a coefficient of reserve whose value should not be less than 10 in order to ensure safe conditions for the transfer of the results of toxicologic studies to public health practice.

## Cognitive Possibilities of Modeling in the Study of Environmental Chemical Pollution

Experimental models are extensively used for the study of toxic effects of environmental chemicals on public health. One of the most important goals of modeling is the prediction of safe levels (maximal permissible concentration, MPC) of toxic substances for the human population. Long-term animal experiments are used as models to establish the hygienic standards and MPC's.

Reliability of modeling results depends on selection of a model with consideration of its cognitive possibility limits, methods used in investigation of modeling process in animals, and conditions of extrapolation of data obtained from animals to humans.

As many authors have pointed out, the animal model is of greater value when the experimental animal is more similar in metabolism to man, but

such information cannot be successfully obtained in all cases. Moreover, criteria for similarity have been insufficiently elaborated. We assume that for similarity criterion a complex of indices must be used: biological parameters of systems reacting on introduction of toxins; quantitative indices of toxicometry, established on the basis of dose - time - effect relations; and characteristics of metabolism.

Based on these indices, there is no single species of animal that is an exact copy of the human. Only mammals are, to a certain degree, similar. Specifically, we found that toxicity indices of various substances for vertebrates are the most similar in human and other species of mammals (Fig. 1).

However, the social nature of man limits the cognitive possibilities of intoxication modeling. In experiments on animals it is impossible to reproduce and to investigate the role of social factors in the change of the toxicity of substances.

Therefore, in the final stage of toxicological investigations, the problem of extrapolating experimental data from a relatively simple model (laboratory animal) to a more complex system (human organism) inevitably emerges. For the

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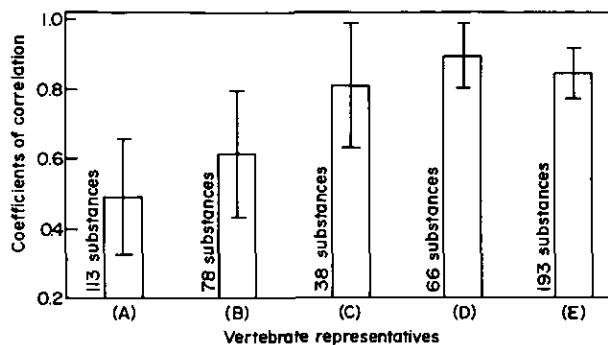


FIGURE 1. Coefficients of correlation between the toxicity indices of chemical substances for the rat and some of the representatives of the lower and higher forms of vertebrates: (A) carp, perch, trout; (B) frog; (C) pigeon, chicken; (D) cat; (E) man.

solution of this problem, extrapolation or "transition" coefficients are used, which, it is assumed, characterize the quantitative correlations of sensitivity between an "average" man and laboratory animals with respect to the effect of chemical substances.

At the same time, results obtained during animal experiments are not representative, since the MPC established on the basis of the test animals may not represent the entire human population. Toxicological investigations are carried out in standard laboratory conditions with the use of homogeneous groups of animals, while the human population is far from being homogeneous in its composition.

In reality, the potential danger of chemical pollution of the environment to public health depends on a multiplicity of factors including the intermittent action of substances, the process of their destruction, and their tendency to accumulate in fish, plants, and other lower organisms. This process is a complex, multicomponent system. In order to determine the comparative significance of these factors, it is necessary to use the theoretical principles of two methods: systems analysis and modeling.

Any model is a simplification of the studied class of phenomena. It makes possible the breakdown of a complex phenomenon into separate components or blocks. Under natural conditions a direct analysis of cause-and-effect chains is very difficult—and sometimes impossible—due to the large number of additional variables.

This complex system in a particular case such as pollution of water by a chemical can be represented by a simpler diagram (Fig. 2).

Even in the simplified form the diagram is fairly complex. In addition, beyond the frames of this

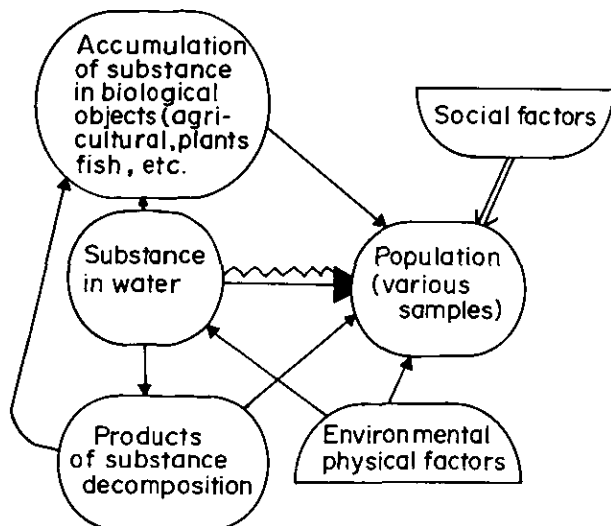


FIGURE 2. Diagram showing the effect of chemical water pollutants on the health of a population.

block diagram there are additional factors which need analysis. Chronic experiment serves as an analog of a nodal cause-and-effect chain linking the compound in the water and health of a population, but this is only one of the subsystems of a multicomponent system. The cognitive possibilities of a chronic intoxication model are limited not only by the heterogeneity of the original population but also in respect to many supplementary factors which vary the degree of toxicity of substances.

The meaning and value of the model can be increased if it is considered as a particular case of a more extensive class of phenomena. This necessitates taking into account supplementary factors, which in turn requires that other models be created in order to establish supplementary "corrective" coefficients. Corrective coefficients along with the extrapolation coefficient must be used for the transfer of experimental data to health practices.

But how do we find the values constituting this "common" transition coefficient? We shall investigate in succession the methodological procedures and possible approaches to determining the separate values which form the "common" coefficient.

## Methods of Extrapolation of Toxicological Data from Animals to Humans

In investigations of the transfer of experimental results to man, extrapolation coefficients are used.

Their real values are selected empirically and vary from 1 (communal hygiene) to 100–500 (nutrition hygiene).

In our investigations, a method of computing extrapolation was developed based on the establishment of a linear relationship between toxicity indices of compounds for various animal species and their body weight. This relation had the characteristics of a general biological regularity (allometric correlations) intrinsic to higher species of animals.

In particular, the indices of the relative weight of the internal organs of mammals decreased regularly starting with the small animals and proceeding to the large. In plotting the initial data on a double logarithmic graph, the points were located along straight lines (Fig. 3).

The physiological constants of mammals (pulse rate, breathing rate, and the consumption of oxygen, food, water, and air) also decreased linearly as a function of the increasing size of the animals (Figs. 4 and 5). The linear relationship with body weight was also characteristic of the indices of the microsomal activity of the liver enzymes (Fig. 6). Phosphatase activity did not differ much for the large or small animals (special case of rectilinear regression).

Using the straight line equation, it was possible to describe adequately the changes of many of the constants of the blood (methionine, glutathione, glucose content, and cholinesterase activity among

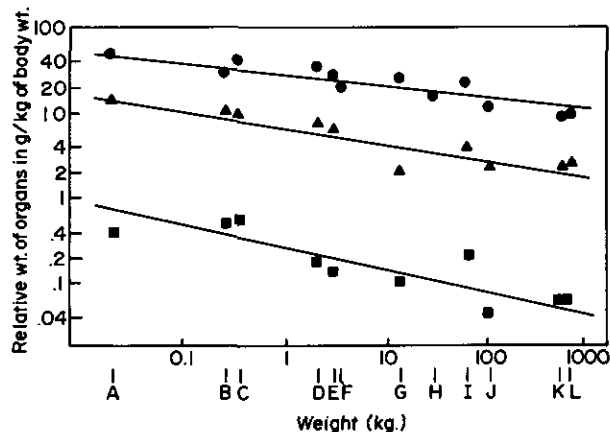


FIGURE 3. Lines of regression for the relative weight of some mammalian internal organs. (●) liver  $r = 0.90$ ,  $b = 0.77$ ; (▲) kidneys  $r = 0.97$ ,  $b = 0.66$  (■) adrenals  $r = 0.87$ ,  $b = 0.58$ . Animals: (A) mouse, (B) rat; (C) guinea pig; (D) cat; (E) monkey; (F) rabbit; (G) dog; (H) small, hoofed, domestic animals; (I) man; (J) pig; (K) large cattle; (L) horse.

others) as a function of increase in body weight of mammals. This trend also held for the duration of pregnancy, the number of simultaneously born offspring, latent period of tumor manifestation, nerve and muscle cell dimension, maturation time of bone marrow cellular elements, duration of erythrocyte life, and a number of other physiological constants which refer to the respiratory and cardiovascular systems. However, the indices of the leukocytarian profile of the blood and of the vitamin B<sub>12</sub> content of plasma varied in the animals without any relationship to body weight.

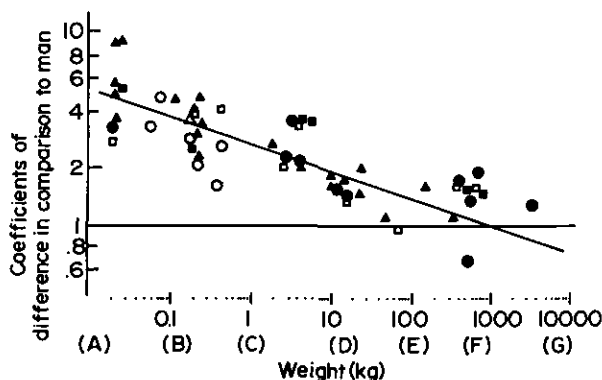


FIGURE 4. Relative indices for the consumption of water by mammals (conversion to kilograms of body weight in comparison to man, with man taken as unity): (A) mouse, hamster; (B) rat, guinea pig, gopher, polecat; (C) rabbit, cat, monkey, fox; (D) dog, sheep; (E) man, pig; (F) horse, cow; (G) elephant.

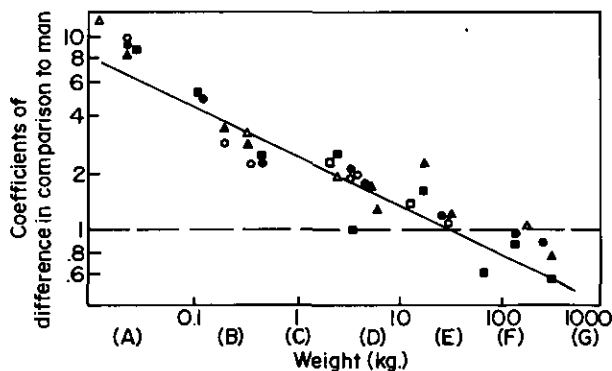


FIGURE 5. Relative indices for the tidal volume in mammals (in cm<sup>3</sup> kg/min in comparison to man, with man taken as unity): (A) mouse; (B) rat; (C) guinea pig; (D) rabbit, cat, monkey; (E) dog, sheep; (F) man, pig; (G) cow, horse.

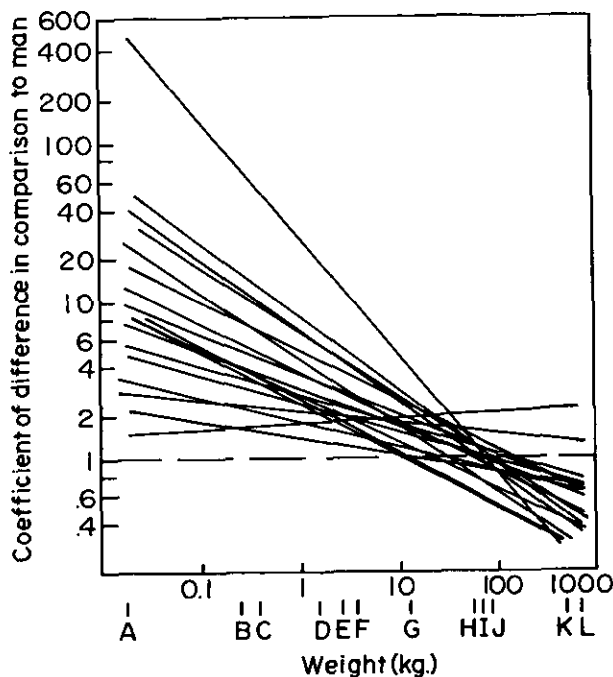


FIGURE 6. Regression lines for enzyme activity of the liver of mammals (in units of activity per kilogram of body weight): (A) mouse; (B) rat; (C) guinea pig; (D) cat; (E) rabbit; (F) monkey; (G) dog; (H) domestic hoofed animals; (I) pig; (J) man; (K) cattle; (L) horse. Enzymes studied were (top to bottom): aminopyrine demethylase; aniline hydroxylase, cytochrome P450; 3-dihydrobutylate dehydrogenase; NAD dehydrogenase; diaminooxidase; cytochrome C; phenylalanine pyruvate oxidase; pyruvate oxidase,  $O_2$  consumption by liver; cytochrome oxidase; succinic dehydrogenase; mitochondrial protein; ornithine transcarbamylase; monoamine oxidase; phosphatase.

It is of interest that the average life span for 70 species of mammals was also linearly related to body weight (Fig. 7).

The linear logarithmic relationship to body weight was established for more than 100 of the most diverse mammalian parameters. These observations are in agreement with other research results (1-4). It was noted in these works that the parameters of energy and water exchange, the dimensions of the blood vessels, and the body length to width ratio were in a linear correlation with mammalian body weight.

Thus we had all the bases for attributing to these relationships a general biological regularity. We designated this the rule determining body weight and gave it the following formulation: the logarithms of mammalian biological parameters are in a linear correlation with the logarithms of

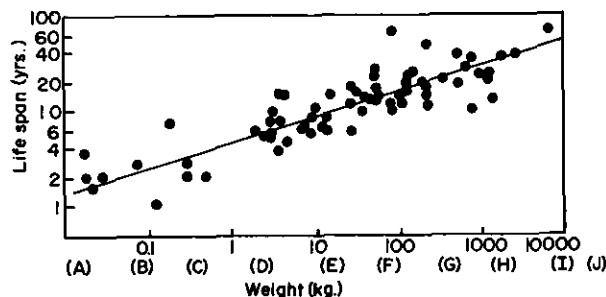


FIGURE 7. Variation of the average life span of some mammals with increased body weight: (A) shrew, mouse (domestic), mouse (field), flying mouse; (B) chipmunk, golden hamster; (C) squirrel, rat, hedgehog, guinea pig; (D) skunk, agouti, jackal, porcupine, rabbit, cat, rhesus monkey, raccoon, polar fox, mongoose, marmot, common hare, lynx; (E) coyote, badger, civet, cat, tapir, nutria, dog, gupeard, wolf, reindeer, goat, jaguar, seal, baboon; (F) antelope, dolphin, sheep, leopard, chimpanzee, hyena, fallow deer, man, llama, alpaca, puma, orangoutang; (G) pig, lion, wild boar, grizzly bear, donkey, deer, tiger, gorilla, zebra; (H) moose, camel, buffalo (asiatic), buffalo (African), polar bear, horse, bull, bison, giraffe, hippopotamus; (I) rhinoceros, elephant; (J) whale.

body weight. This rule is expressed by the parabolic function  $x = ay^B$  or by the straight line equation:

$$\log x = \log a + b \log y$$

The linear correlation between the indices of toxicity and the body weight of various animal species was a particular case of this body weight rule. In analyzing the relationship between species differences and the action of several hundred chemical compounds, it was shown that the regularities of the comparative sensitivity of the animals to 80-85% of the substances can be characterized by a straight line equation. Figure 8 depicts all of the practically possible variants of the changes in the toxicity parameters of substances (in mg/kg) for mammals as a function of body weight.

The problem of dealing with the possible difference in sensitivity among animals of the same species but different body weights has been discussed in a number of works (5, 6).

Gori's study (7) indicated that the relationship between the sensitivity of animals and body weight is to a great extent characteristic primarily for various species of mammals.

In verification research we attempted to use the derived regression equations to determine theoretically (by the computational method) the values of the biological constants and the toxicity indices of substances for various mammals. The computed lethal dose of 34 substances for dogs as

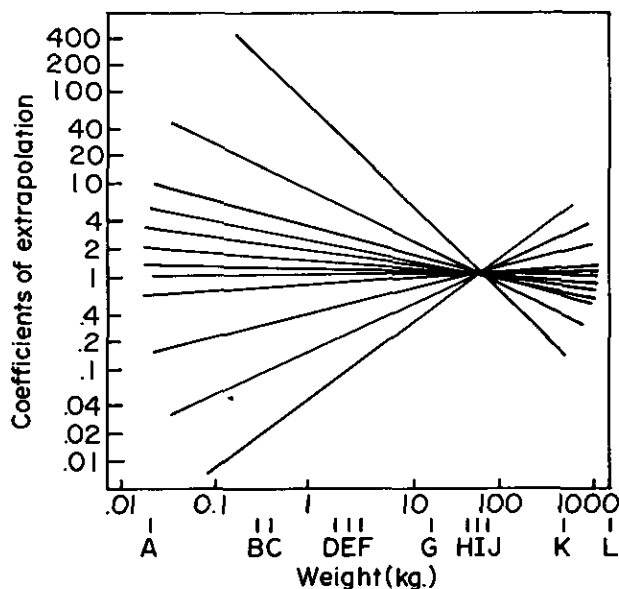


FIGURE 8. Flabelliform of species differences of mammals with respect to the action of toxic substances: (A) mouse; (B) rat; (C) guinea pig; (D) cat; (E) rabbit; (F) monkey; (G) dog; (H) small hoofed animals; (I) man; (J) pig; (K) horse; (L) large, hoofed animals.

determined by regression analysis of the toxicity indices for four species of small laboratory animals differed on the average from the empirically determined values by 1.2–0.2 times. The maximum limits of divergence for the individual values (taking into consideration the second sigmas) reached 3.4 times (left part of Fig. 9).

Further, we attempted to “compute” man, i.e., to determine theoretically the biologic constant values for mammals with a body weight of 70 kg. This was done on the basis of corresponding biological indices for animals. In deriving these calculations 86 regression equations were used. These equations had been derived earlier during the substantiation of the body weight rule. When the computed values were compared to the actual values for man, the results differed in one or the other direction by not more than 1.2–1.5 times, while the maximum limits of divergence for the individual values did not exceed 2.0–2.5 times (right part of Fig. 9). Only in the case of four biological parameters did the computed values not agree with the actual values. Under these conditions, the life span indices for man, the relative weight of the brain and the amount of oxygen consumed by the brain exceeded the computed values by 4.7, 8.8, and 11.2 times, respectively, and man lagged behind the animals (index of the physical development of

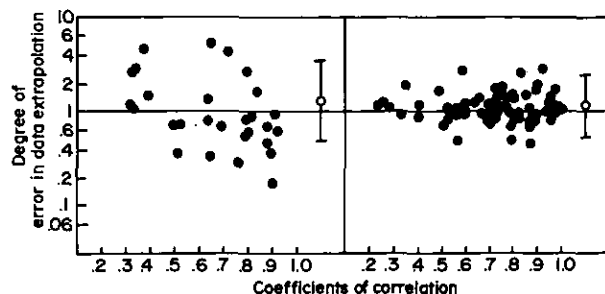


FIGURE 9. Evaluating the accuracy of computational data. Comparison of (left) toxicity parameters for dogs and (right) biological parameters for man, parameters obtained from regression equations. Correlation between parameters and animal weight.

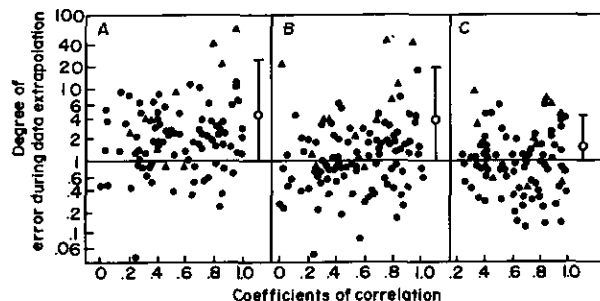


FIGURE 10. Degree of reliability of various methods for extrapolating the data of toxicological experiments from animals to man by using the indices of acute toxicity: (A) conditions of extrapolation from white rats; (B) conditions of extrapolation from the most sensitive species of laboratory animals; (C) extrapolation, taking into consideration the values of the coefficients of regression B.

children) with respect to the body weight doubling period by 7.7 times. It is readily apparent that these exceptions reflect the socially conditioned indices which differentiate man from the animals. At the same time, a large number of values for man coincided with remarkable accuracy with those calculated for the 70 kg animal.

Finally, to verify the possibility of using the regression equations for the extrapolation of toxicologic data from animals to man, this method was compared to others being used in practice or recommended in the literature. These methods were used in processing the information which we collected on the acute toxicity indices of 107 substances for man and for four to six laboratory animal species. The limits of divergence between the extrapolated and the actual values of the toxicity indices of substances in the case of man are

Table 1. Error values permissible in various extrapolation methods.

Statistical parameters	Direct transition of experimental data		Recomputation to body surface (from white rats)	According to regression equation	Method of Ulanova and Van Nordwijk
	From white rats	From the most sensitive species of animals			
$\bar{x} \pm m$	$5.5 \pm 2.1$	$4.3 \pm 1.5$	$1.1 \pm 1.2$	$1.5 \pm 0.2$	$2.2 \pm 0.4$
$2b$	40.1	31.7	23.3	3.4	2.1
$n$	95	107	95	89	30

given in Figure 10. Table 1 contains the results of the mathematical processing of the obtained data for determining the values of errors which occur with various methods of extrapolation.

The error values given in Table 1 show that the use of the method of direct transfer of data from white rats to man results in an exaggeration of the results from 5.5 to 40 times. With direct data transfer from more sensitive species of animals, accuracy increased noticeably; the error exceeded 30 times only for vegetotropic compounds.

The method for calculating substance doses per unit of body surface for half of the analyzed compounds turned out to be sufficiently reliable and led to the leveling of the differences in sensitivity of humans and animals. For 4% of the substances this method caused exaggerated results (for atropine by 34 times), but 46% of the substances produced lower results (for example, dichloroethane 10 times, aldrin 17 times, and ANTU 87 times). The methodological conditions associated with this method must be established more specifically in order to improve accuracy in recomputing doses to body surface.

The most reliable and accurate extrapolation methods are those based on preliminary investigation of the species differences of animals and the effect of the studied substances.

Both the method of direct transfer on the basis of establishing identical sensitivity of laboratory animal species to the investigated substance recommended by Van Nordwijk (8) and by Ulanova (9) and the computation method of extrapolation developed by us permit the prediction of the toxicity parameters of substances for humans with a sufficiently high degree of accuracy. The permissible error by these methods, even for individual substances, does not exceed 3–4 times. It is noteworthy, however, that the methods of Van Nordwijk and Ulanova could be applied only for 25–35% of the substances. The extrapolation method with the use of a regression equation has great possibilities and is applicable to 80–85% of the substances.

In order to calculate the extrapolation coefficient, it is necessary to determine the indices of species sensitivity with respect to the studied substances ( $LD_{50}$ , or the probable single exposure threshold values) under experimental conditions for four species of laboratory animals. Then we must find out whether the logarithms of the determined values correlate with the logarithms for the body weight of animals, and further we must derive a general type rectilinear regression equation,  $\log H = \log a + \log W$ , which is used to determine the toxicity parameter of a substance  $H$  for man ( $W = 70$  kg). Finally we must determine the coefficient of extrapolation  $K = H$  by using the relationship of this value to the analogous indices of toxicity of a studied substance for the same species used in the chronic experiment.

Thus, using optimal extrapolation methods based on the preliminary study of the regularities associated with the species sensitivity of animals, the transfer error of experimental data for the "average" human will not be greater than 3–4 times. Consequently, to increase extrapolation reliability it is necessary to take into account the possible error and for this purpose to use a supplementary "reserve" coefficient, corresponding to the maximal value of the given error that is equal to 4.

## Significance of Methodological Conditions in Conducting Experiments

We have examined one of the extrapolation conditions which should be considered in the final stage of investigation. However, we should remember that experimental data can accurately be transferred only when exact toxicity parameters are established for the substances in the animal experiments.

Analysis of Soviet results on substantiation of hygienic standards of harmful substances in reservoir water shows that the threshold doses of the

same substance established by various methods can differ within the range of 15–70 times.

As we know, for each substance under study there can be as many threshold doses as the number of physiological functions of an organism which a given toxin can disturb. This is one of the basic principles for establishing reliable toxicity parameters and has been strictly observed in the development of health standards in the past. It is sufficient to say that of 400 recommended MPC's for reservoir water, 60% of the standards were based on the highly sensitive method of conditioned reflexes.

To substantiate the experiment duration, the human to animal life span relationship is usually considered. Thus, for example, it is assumed that 6 months of a rat's life corresponds to 9–14 years of human life. However, it is necessary to introduce corrective factors into these computations.

On the basis of the body weight rule, we found that the average life span of 70 species of mammals follows a linear correlation with their body weight ( $r = 0.833$ ). The regression equation expressing this relationship is as follows:  $\log x = 0.719 - 0.252 \log W$ . The average human life span, as a socially conditioned index, was an exception to the rule. But the given equation permits the calculation of the average longevity for representatives of mammals with a body weight of 70 kg, which is human weight.

## Variability Limits of the Sensitivity of Various Subgroups of a Population to the Effect of Chemical Compounds

The level of environmental chemical pollution determined by animal experiments to be harmless for an "average" human must be equally safe for all subgroups of the population, including children, the aged, and persons with various chronic diseases.

Each individual is different from other persons by his sensitivity to toxins. Sixfold differences in the individual sensitivity of children were observed with respect to the action of fluorine and nitrates in the drinking water. Our investigations established that the sensitivity of six adult humans to the effect of acotophosis varied as much as 4–6 fold. An approximately similar degree of variability is also characteristic for animals. For 50 compounds, individual differences of sensitivity in animals, according to indices  $LD_{50}/LD_{15}$ , were 3–5 times, and

according to the ratio  $LD_{100}/LD_0$  differences were  $5.9 \pm 0.4$  for mice and  $4.2 \pm 0.4$  for rats.

Age differences produce similar variations. According to a number of studies, the sensitivity of children to certain compounds is 3–5 times that of adults. Generalized data on animal sensitivity to 119 substances suggest that the ratio of differences of young and adult organisms is  $2.4 \pm 0.23$ , while the old animals are  $2.4 \pm 0.31$  times as sensitive than the adults.

The practical coincidence of the limits of the individual and age variability suggests that experimental models can be used for evaluating the role of biological factors which affect the sensitivity to chemical compounds.

Limits of sexual differences of white rats with respect to the effect of 149 substances did not exceed 2–3 times and only to certain phosphoro-organic compounds were the females 3–4 times more sensitive than males.

Modeling of animal chronic epilepsy, hepatitis, nephritis, toxic myocarditis led to increasing their sensitivity to toxins 2–5 times. This problem deserves particular attention, and many aspects remain unclear. The nature and the degree of toxicity variation in relation to pathology types has not been established. The increased vulnerability of population samples suffering from chronic disorders must be considered in studying the problem of the reliability of experimentally established health standards.

The result was 15.1 years (13.8–16.8 years). This figure can be designated as a biologically conditioned life span of man. Another method of calculation (based on Rubner's constants for mammals) gives a similar number: 15–17 years. Therefore, the average life span of the rat (approximately 2.5 years) corresponds only to 15–17 years of a human life. For this reason, in order to find the smallest threshold dose, a complex must be used of the most suitable research methods with respect to the disturbed functions of an organism in conjunction with integrated (nonspecific) tests. Thus we begin to understand the difficulties associated with reproducing in animals such diseases as arteriosclerosis, myocardial infarction and others, which mainly develop in old age, i.e., during the period of socially extended human life span.

The disproportionality in the life spans of animals and humans must be considered in the experimental study of the long-term effects of substances and in the substantiation of extrapolation of the results to man. For example, in the case of carcinogenic doses which can cause effects only

beyond the human life span, the proportional time period for an experimental model must correspond to at least 5–6 times the life span of animals.

The nonlinearity of the time parameters of the model and the original is equally significant in selecting the time for conducting chronic toxicological experiments. There is no strict scientific basis for determining the optimal duration of observation of the effects of substances introduced daily into animal organisms. Recommendations vary from 3–6 to 12 months. In the case of substances with cumulative properties, intensification of the toxic effect is characteristic during the course of several months of observation. Obviously, more complete information on the toxic properties of such substances can be obtained only in a lifelong experiment on the animal, in which case the overall duration of research increases sharply.

For this reason it is necessary to compare the isoeffective doses of substances computed on the basis of the dose–effect–time relationship for various periods of intoxication (1–2 weeks, 1, 6, and 12 months, and end of life of the animals). As a result of these studies, it will become possible to determine the transfer coefficients (safety factors). On the basis of these, the data obtained during the course of a 3–6 month intoxication can be extrapolated to a longer period of time, i.e., to the end of life of the animals.

A series of other factors has less effect on the change of sensitivity of an organism to toxins (type of feeding, the role of seasonal and circadian rhythms, and, in conditions of hot climate, the toxicity of substances contained in water, which increases up to 3 times). The significance of these factors has not yet been studied in detail under various experimental conditions (introduction of drugs, vaccination, pregnancy, hypodynamia, etc.).

The complexity of social factors such as the role of living conditions connected with urbanization is impossible to study in animal experiments.

Thus in determining the conditions for the transfer of toxicologic data into health practice, it is first necessary to correct the experimental results, bearing in mind that the health of all of the population samples must be protected. The present level of knowledge does not permit the accurate determination of the magnitude of a supplementary correction coefficient. In any case, this coefficient must cover variability limits of the population contingents most sensitive to toxins. In this manner the role of the remaining lesser factors will be leveled. It is obvious that in selecting this type of coefficient we must first take into consideration the

limits of the individual variability of a population and the increased sensitivity to toxins of people with chronic disorders. The variability limits of these population groups must be considered, and then the correction coefficient value, evidently, must be not less than 6.

## Conclusion

Results of experimental studies obtained on chronic intoxication models must be carefully transferred into health practice. For the purpose of adequate safety margins in extrapolation of toxicological data extrapolated from animals to man, it is expedient to use appropriate safety factors. This methodological approach will serve to increase the health significance of the chronic intoxication and will make it possible to establish suitable hygienic standards for harmful substances on the basis of animal experiments.

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